Carbohydrate-Based Cocktails that Decrease the Population of *Salmonella* and *Campylobacter* in the Crop of Broiler Chickens Subjected to Feed Withdrawal

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ABSTRACT The efficacy of various carbohydrate-based cocktails in reducing the number of enteropathogens in the crops of broilers subjected to feed withdrawal was examined. Market-aged broilers that had been orally challenged with *Salmonella typhimurium* were provided the cocktails during a 12-h feed withdrawal. After feed withdrawal, the broilers were processed, and their crops were aseptically removed and weighed. Crops were then blended in distilled water, and the pH of the suspensions was measured electronically. Populations of *S. typhimurium*, *Campylobacter*, and lactic acid bacteria in the crop suspensions were enumerated. Findings indicated that significantly fewer *S. typhimurium* and *Campylobacter* were recovered from the crops of broilers that had been provided cocktails supplemented with sucrose than from the crops of broilers provided cocktails supplemented with equal concentrations (wt/vol) of glucose. Furthermore, significantly fewer *S. typhimurium* were recovered from the crops of broilers provided cocktails supplemented with 2 to 10% sucrose than from the crops of broilers provided water or cocktails that were not supplemented with carbohydrates. The pH of the crop contents of broilers provided carbohydrate cocktails were lower than the pH of the crops of broilers provided water or cocktails that were not supplemented with carbohydrates. Consumption of the cocktails did not produce significant changes in the crop weights. Findings indicate that altering the composition of carbohydrate-based cocktails provided to broilers during feed withdrawal may affect the efficacy of cocktails in reducing the number of enteropathogens recovered from the crops of broilers.

(*Key words*: crop, carbohydrate, feed withdrawal, *Salmonella*, *Campylobacter*)

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INTRODUCTION

The ability of the native bacterial flora of the crop of market-age broilers to limit the growth of foodborne pathogens diminishes during feed withdrawal (Ramirez et al., 1997; Byrd et al., 1998; Hinton et al., 1999b). When broilers consume feed, beneficial crop bacteria ferment ingested contents to produce significant concentrations of lactic, acetic, and propionic acids (Hinton et al., 1992a,b; Corrier et al., 1999; Hinton et al., 1999). Higher concentrations of acidic, bacteriostatic, or bacterial metabolic products in the crop contents of broilers are associated with reduced crop pH and lower populations of enteropathogens in the crop. Crops, however, empty of ingesta within 6 h after broilers are denied access to feed (Wabeck, 1972; Hinton et al., 2000b). Reductions in the levels of fermentable carbohydrates available in the crop during feed withdrawal are followed by decreases in the population of crop lactic acid bacteria; decreases in the concentration of acidic, fermentation products; increases in the pH of the crop contents; and a reduction of the ability of the crop to inhibit the growth of enteropathogens.

The ability of the crop to inhibit the growth of foodborne pathogens can be maintained during feed withdrawal by providing broilers a glucose-based cocktail after the broilers are denied access to feed (Hinton et al., 2000a). The cocktail provides fermentative, native crop bacteria with a carbohydrate that can be converted into acidic products (Hinton et al., 1992a) and with nutrients that are required to support the growth of these beneficial bacteria. Fermentative bacteria can metabolize a wide range of carbohydrates and metabolic intermediates other than glucose to produce substances that can inhibit the growth of enteropathogens, however. Therefore, the purpose of the present study was to compare the efficacy of glucose- or sucrose-based cocktails in reducing the number of *Salmonella* and *Campylobacter* in the crops of broilers provided the cocktails during feed withdrawal.

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Abbreviation Key: BGS = BG sulfa.

MATERIALS AND METHODS

Husbandry

On Day 1, 5-wk-old broilers were obtained from local commercial growers and transported to a poultry housing facility. Broilers were obtained from different flocks for each trial. All broilers were orally challenged with 10^9 cfu of a nalidixic-acid-resistant *Salmonella typhimurium* strain ST-10 and were divided into five treatment groups of six broilers each. Treatment groups were placed in separate pens with wood shavings on the floor. Feed and water were provided ad libitum under continuous lighting. Feed was a corn-soybean meal, pelleted grower ration (3,200 ME/kg and 19% CP). Water was provided in bell shaped drinkers. On Day 2 (24 h after the first *S. typhimurium* challenge), a second oral challenge of 10^9 cfu of *S. typhimurium* was administered to each broiler.

Cocktail Preparation and Feed Withdrawal

Basal feed withdrawal cocktails were prepared that contained (g/L) proteose peptone,[2] 10; beef extract,[2] 5; yeast extract,[2] 5; polyoxyethylene-sorbitan monooleate (Tween 80),[3] 1; magnesium sulfate, heptahydrate,[3] 0.5; and manganese sulfate, monohydrate,[3] 0.2. Basal cocktails were supplemented with appropriate concentrations (wt/vol) of D- (+)-glucose[3] or sucrose.[3] The pH of all cocktails was adjusted to 6.0 with a solution of three parts 1 *M* acetic acid, glacial[3] and one part 1 *M* propionic acid.[3] Cocktails were autoclaved at 121 C for 15 min and then stored at 4 C until used.

Feed withdrawal was initiated by removing feeders from pens on Day 2, 12 h after the second *S. typhimurium* challenge and 12 h before the broilers were to be processed on Day 3. The appropriate waterers were emptied 4 h before feeders were removed from the pens, and plastic tubing was used to connect the waterers to carboys containing the cocktails. After broilers had been provided the cocktail or water with access to feed for 4 h, feed was removed and the 12-h feed withdrawal period began. In Trial 1, broilers were provided cocktails supplemented with 1) no carbohydrate, 2) 4% glucose, or 3) 2 or 4% sucrose. In Trial 2, broilers were provided cocktails supplemented with 4, 6, 8, or 10% sucrose. Control groups of broilers were provided water instead of cocktails during feed withdrawal in both trials.

Collection and Analysis of Crops

At the end of the feed withdrawal period, each group of broilers was placed in separate coops and transported to a pilot plant poultry processing facility. For each trial, broilers from different treatment groups were processed separately by electrocution, hot water scalding, and mechanical picking. Crops were aseptically removed from the processed carcasses, and five of the six crops were selected for further analysis. Each selected crop was placed in a separate sterile plastic bag,[4] weighed, and blended with 20 mL of sterile distilled water in a Stomacher Lab-Blender[5] for 1 min at normal speed. The pH of the suspensions were measured electronically.[6] Crop suspensions were serially diluted in 0.1% peptone and spread on the appropriate bacteriological medium.

Lactic acid bacteria were enumerated on lactic acid bacteria agar (Atlas, 1993). Inoculated lactic acid bacteria agar plates were incubated anaerobically in a controlled environment chamber[7] at 35 C for 48 h. *S. typhimurium* were enumerated by plating crop suspensions on BG sulfa[8] (BGS) agar supplemented with 150 μg/mL of nalidixic acid[6] and 25 μg/mL novobiocin.[9] BGS plates were incubated aerobically at 35 C for 18 to 24 h. Salmonellae-like colonies from BGS plates were confirmed as *S. typhimurium* by biochemical tests with triple sugar iron agar[2] and lysine iron agar[2] and by serological tests with *Salmonella* O antiserum Poly A[2] and *Salmonella* O Antiserum Group B, Factors 1, 4, 5, 12.[2] *Campylobacter* were enumerated on *Campylobacter* agar, Blaser.[9] Inoculated *Campylobacter* plates were incubated for 48 h at 42 C under microaerophilic conditions produced by an activated BBL CampyPak Plus[8] gas generator envelope in a BBL GasPak Jar System. Campylobacter-like colonies were confirmed with the Latex-CAMPY(jcl)J *Campylobacter* culture confirmation test.[9] Direct plating on each medium could detect as few as 20 cfu/mL.

Portions of the crop suspensions were also placed in enrichment bacteriological media to detect lower levels (<20 cfu/mL) of *Salmonella* and *Campylobacter*. *Salmonella* enrichment was performed by adding 1 mL of the crop suspension to 10 mL of universal preenrichment broth.[10] Inoculated tubes were incubated aerobically at 35 C for 24 h. Contents of the incubated enrichment cultures were streaked on BGS supplemented with nalidixic acid and novobiocin. The streaked plates were incubated aerobically at 35 C for 18 to 24 h, and salmonellae-like colonies were confirmed as *S. typhimurium* by using biochemical and serological tests described above. *Campylobacter* enrichments consisted of transferring 1 mL of the crop suspensions to tubes containing 10 mL of *Campylobacter* enrichment broth.[7] Inoculated tubes were incubated at 42 C under microaerophilic conditions in BBL GasPak Jar Systems with BBL CampyPak Plus gas generator envelopes. Contents of the enrichment tubes were streaked on *Campylobacter* agar, Blaser; and the plates were incubated at 42 C under microaerophilic conditions. Campylobacter-like colonies were confirmed as *Campylobacter* by using methods described above.

Statistical Analyses

Group means (five broiler crops per treatment) of data for each trial were compared to determine significant

[2]Difco Laboratories, Detroit, MI 48232.
[3]Sigma Chemical Co., St. Louis, MO 63178.
[4]Tekmar, Cincinnati, OH 45249.
[5]Seward Medical Ltd., London SE1 1PP, U. K.
[6]Fisher Acumet Meter, Pittsburgh, PA 15205.
[7]Coy Laboratory Products, Inc., Grass Lake, MI 49240.
[8]Becton Dickinson and Co., Sparks, MD 21152.
[9]Integrated Diagnostics, Inc. Baltimore, MD 21227.
[10]Acumedia Manufacturers, Inc., Baltimore, MD 21211.

TABLE 1. Comparisons of weight, pH, and population of lactic acid bacteria, *Salmonella typhimurium*, and *Campylobacter* of the crop of broilers that were denied access to feed and provided cocktails supplemented with carbohydrates

Carbohydrate added to basal cocktail[1]	Crop weight (g)[2]	Crop pH[2]	Log cfu of crop bacteria/g tissue[2]			Number of and percentage of positive crops after enrichment	
			Lactic acid bacteria	*S. typhimurium*	*Campylobacter*	*S. typhimurium*	*Campylobacter*
Water (control)	7.54 ± 1.64[a]	6.73 ± 0.06	6.75 ± 0.60[a]	1.37 ± 1.29[a]	7.31 ± 0.12[a]	5 of 5 (100%)	5 of 5 (100%)
None	7.23 ± 1.27[a]	6.81 ± 0.17	6.70 ± 0.46[a]	2.05 ± 1.22[a]	7.61 ± 0.48[a]	5 of 5 (100%)	5 of 5 (100%)
2% sucrose	7.27 ± 1.05[a]	6.38 ± 0.17	7.12 ± 0.50[a]	0.90 ± 1.34[a]	7.38 ± 0.05[a]	5 of 5 (100%)	5 of 5 (100%)
4% sucrose	6.90 ± 0.87[a]	6.38 ± 0.23	7.03 ± 0.88[a]	0.00 ± 0.00[b]	4.22 ± 3.87[b]	5 of 5 (100%)	2 of 5 (40%)
4% glucose	7.11 ± 1.21[a]	6.40 ± 0.32	7.22 ± 0.32[a]	0.32 ± 0.71[a]	6.98 ± 0.59[a]	5 of 5 (100%)	5 of 5 (100%)

[a,b]Different superscripts within columns indicate significant differences between values from crops of broilers provided water (controls) and broilers provided basal or carbohydrate cocktail during feed withdrawal.

[1]Weight/volume.

[2]Values are means ± standard deviations; n = 5.

differences among experimental data. Data were analyzed using GraphPad InStat version 3.00 for Windows 95[11] to perform one-way ANOVA. When ANOVA detected significant differences among group means, Dunnett's test was used to determine which of the treatment groups differed significantly from the control group. All significant differences were determined at $P < 0.05$.

RESULTS

Trial 1

Results from Trial 1 indicated that the type and concentration of carbohydrate used to supplement cocktails provided to broilers during feed withdrawal could influence the ability of the crop to maintain its natural ability to inhibit the growth of foodborne pathogens (Table 1). The crop weights of broilers provided the basal cocktail and carbohydrate cocktails did not differ significantly from the crop weights of broilers provided water during feed withdrawal. The pH of the crops of broilers provided carbohydrate cocktails were approximately 0.3 to 0.4 U lower than the pH of the crops of broilers provided water or the basal cocktail, although neither the crops of broilers provided the basal cocktail nor the carbohydrate cocktails contained significantly more lactic acid bacteria than the crops of broilers that were provided water. Significantly fewer *S. typhimurium* and *Campylobacter* were recovered from the crops of broilers provided the cocktail supplemented with 4% sucrose than from the crops of broilers provided water during feed withdrawal, but there was no significant difference in the number of these enteropathogens recovered from crops of control broilers and broilers provided cocktails supplemented with no carbohydrate, 2% sucrose, or 4% glucose. Furthermore, after enrichment procedures no *Campylobacter* were recovered from the crops of three of five of the broilers provided the 4% sucrose cocktail, whereas the enrichment procedure recovered *S. typhimurium* and *Campylobacter* from the crops of each of the broilers in the control group and other treatment groups in this trial.

Trial 2

Providing broilers the basal cocktail supplemented with several concentrations of sucrose significantly reduced the number of salmonellae recovered from the crops of broilers (Table 2). The crop weights of broilers provided cocktails supplemented with 4 to 10% sucrose did not differ significantly from crop weights of broilers provided water. Although the pH of the crops of broilers provided the sucrose cocktails were approximately 0.5 U less than the pH of the crops of broilers that were provided water during feed withdrawal, the number of lactic acid bacteria recovered from the crops of broilers provided sucrose cocktails were not significantly greater than the number of lactic acid bacteria recovered from the crops of broilers provided water. Significantly fewer *S. typhimurium* were recovered from the crops of broilers provided each of the sucrose cocktails than from the crops of broilers provided water during feed withdrawal. Although enrichment procedures recovered *S. typhimurium* from the crops of each of the broilers provided water during feed withdrawal and from the crops of four of five of the broilers provided cocktails containing 6, 8, or 10% sucrose, the pathogen was recovered from only one of five of the crops of broilers provided the 4% sucrose cocktail during feed withdrawal. Neither direct plating nor enrichment recovered *Campylobacter* from the crops of any broilers used in Trial 2.

DISCUSSION

These findings indicate that modifying the composition of carbohydrate-based cocktails provided to broilers for 12 h during feed withdrawal influences the effectiveness of cocktails in reducing the population of foodborne pathogens in the crops of the broilers. When broilers are denied access to feed, the crop population of lactic acid bacteria decreases, the crop pH increases (Corrier et al., 1999; Hinton et al., 1999), and the natural ability of the crop to inhibit growth of foodborne pathogens dimin-

[11]GraphPad Software, San Diego, CA 92121.

TABLE 2. Comparisons of weight, pH, and population of lactic acid bacteria, *Salmonella typhimurium*, and *Campylobacter* of the crop of broilers that were denied access to feed and provided cocktails supplemented with sucrose

Cocktail supplement	Crop weight (g)[1]	Crop pH[1]	Log cfu of crop bacteria/g tissue[1]			Number of and percentage of positive crops after enrichment	
			Lactic acid bacteria	*S. typhimurium*	*Campylobacter*	*S. typhimurium*	*Campylobacter*
Water (control)	7.94 ± 1.10[a]	6.52 ± 0.21	7.34 ± 0.49[a]	3.44 ± 0.52[a]	NR[2]	5 of 5 (100%)	NR
4% sucrose	9.18 ± 3.69[a]	5.95 ± 0.57	7.88 ± 0.72[a]	0.00 ± 0.00[b]	NR	1 of 5 (20%)	NR
6% sucrose	7.98 ± 1.72[a]	5.93 ± 0.61	7.21 ± 1.14[a]	0.74 ± 1.06[b]	NR	4 of 5 (80%)	NR
8% sucrose	7.62 ± 1.15[a]	6.06 ± 0.43	8.11 ± 0.37[a]	0.30 ± 0.67[b]	NR	4 of 5 (80%)	NR
10% sucrose	7.62 ± 1.15[a]	6.06 ± 0.43	7.61 ± 1.24[a]	1.24 ± 1.18[b]	NR	4 of 5 (80%)	NR

[a,b]Different superscripts indicate significant differences between values from crops of broilers provided water (controls) and broilers provided basal or carbohydrate cocktail during feed withdrawal.

[1]Values are means ± standard deviations; n = 5.

[2]NR = None recovered.

ishes (Hargis et al., 1995; Ramirez et al., 1997). Providing broilers a carbohydrate-based cocktail during feed withdrawal helps the crop maintain its natural ability to inhibit growth of enteropathogens by providing native lactic acid bacteria (Fuller, 1977; Soerjadi et al., 1981) with substrates required for growth and acid production (Hinton et al., 2000a). Some lactic acid bacteria are able to form slime layers and capsules composed of the polysaccharide dextran when grown on medium supplemented with sucrose but not when grown on medium supplemented with glucose (Brock et al., 1994; Edwards et al., 2000). The sticky dextran slime layers and capsules may help bacteria attach to surfaces (Wilcox et al., 1993), and these polysaccharides may serve as fermentation substrates for other bacteria. The production and fermentation of dextrans by lactic acid bacteria in the crops of broilers provided the sucrose-based cocktails might have been one of the factors that increased the effectiveness of sucrose-based cocktails when compared to the glucose cocktail in reducing crop contamination by enteropathogens. Other trials have indicated that supplementing cocktails with carbohydrate levels greater than the optimal concentration required for reduction of the enteropathogen population results in higher recovery of enteropathogens from the crop (Hinton et al., 2000a). Recovery of higher populations of enteropathogens from the crops of broilers provided cocktails supplemented with higher than optimal concentrations of carbohydrates may be due to enteropathogen growth stimulated by residual carbohydrates that were not metabolized by the lactic acid bacteria that are provided excess substrate.

Consumption of the cocktails did not inhibit the normal evacuation of ingesta from the crop that is desired during feed withdrawal; therefore, providing the proper carbohydrate-based cocktails to broilers during feed withdrawal may reduce the number of foodborne pathogens present while still allowing the crop to empty. Other studies must be conducted to determine if increasing the length of time that the cocktail is administered to broilers will increase the efficacy of sucrose-based cocktails in reducing enteropathogen levels in the alimentary tract of broilers subjected to feed withdrawal.

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REFERENCES

Atlas, R. M. 1993. Handbook of Microbiological Media. CRC Press, Boca Raton, FL.

Brock, T. D., M. T. Madigan, J. M. Martinko, and J. Parker. 1994. Biology of Microorganisms. Prentice Hall, Englewood Cliffs, NJ.

Byrd, J. A., D. E. Corrier, M. E. Hume, R. H. Bailey, L. H. Stanker, and B. M. Hargis. 1998. Effect of feed withdrawal on *Campylobacter* in the crops of market-age broiler chickens. Avian Dis. 42:802–806.

Corrier, D. E., J. A. Byrd, B. M. Hargis, M. E. Hume, R. H. Bailey, and L. H. Stanker. 1999. Survival of *Salmonella* in the crop contents of market-age broilers during feed withdrawal. Avian Dis. 43:453–460.

Edwards, C. G., M. D. Collins, P. A. Lawson, and A. V. Rodriguez. 2000. *Lactobacillus nagelii* sp. nov., an organism isolated from a partially fermented wine. Int. J. Syst. Evol. Microbiol. 50:699–702.

Fuller, R. 1977. The importance of lactobacilli in maintaining normal microbial balance in the crop. Br. Poult. Sci. 18:85–94.

Hargis, B. M., D. J. Caldwell, R. L. Brewer, D. E. Corrier, and J. R. DeLoach. 1995. Evaluation of the chicken crop as a source of *Salmonella* contamination of broiler carcasses. Poult. Sci. 74:1548–1552.

Hinton, A., Jr., R. J. Buhr, M. E. Hume, and K. D. Ingram. 1999. Changes in the concentration of metabolic intermediates in the crop of poultry subjected to feed withdrawal. Poult. Sci. 78(Suppl. 1):27. (Abstr.)

Hinton, A., Jr., R. J. Buhr, and K. D. Ingram. 2000a. Reduction of *Salmonella* in the crop of broiler chickens subjected to feed withdrawal. Poult. Sci. 79:1566–1570.

Hinton, A., Jr., R. J. Buhr, and K. D. Ingram. 2000b. Physical, chemical, and microbiological changes in the crop of broiler chickens subjected to incremental feed withdrawal. Poult. Sci. 79:212–218.

Hinton, A., Jr., D. E. Corrier, and J. R. DeLoach. 1992a. *In vitro* inhibition of *Salmonella typhimurium* and *Escherichia coli* O157:H7 by an anaerobic Gram-positive coccus isolated from the cecal contents of adult chickens. J. Food Prot. 55:162–166.

Hinton, A., Jr., D. E. Corrier, and J. R. DeLoach. 1992b. Inhibition of the growth of *Salmonella typhimurium* and *Escherichia coli* O157:H7 on chicken feed media by bacteria isolated from the intestinal microflora of chickens. J. Food Prot. 55:419–423.

Ramirez, G. A., L. L. Sarlin, D. J. Caldwell, C. R. Yezak, Jr., M. E. Hume, D. E. Corrier, J. R. DeLoach, and B. M. Hargis. 1997. Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. Poult. Sci. 76:654–656.

Soerjadi, A. S., S. M. Stehman, G. H. Snoeyenbos, O. M. Weinack, and C. F. Smyser. 1981. The influence of lactobacilli on the competitive exclusion of paratyphoid salmonellae in chickens. Avian Dis. 25:1027–1033.

Wabeck, C. J. 1972. Feed and water withdrawal time relationship to processing yield and potential fecal contamination of broilers. Poult. Sci. 51:1119–1121.

Wilcox, M. D., R. J. Fitzgerald, B. O. Adams, M. Patrikakis, and K. W. Knox. 1993. Biochemical properties of *Streptococcus sobrinus* reisolates from the gastrointestinal tract of a gnotobiotic rat. J. Gen. Microbiol. 139:929–935.